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Phospho-Smad2-S465/S467 Rabbit mAb

Catalog No.: AP1654 Recombinant

Basic Information

Observed MW
60 kDa

Calculated MW
49 kDa/52 kDa

Category
Primary antibody

Applications
WB,IP,IF/ICC,ChIP,ELISA

Cross-Reactivity
Human, Mouse, Rat

CloneNo number
ARC4171

Background

The protein encoded by this gene belongs to the SMAD, a family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the C. elegans gene Sma. SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways. This protein mediates the signal of the transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation. This protein is recruited to the TGF-beta receptors through its interaction with the SMAD anchor for receptor activation (SARA) protein. In response to TGF-beta signal, this protein is phosphorylated by the TGF-beta receptors. The phosphorylation induces the dissociation of this protein with SARA and the association with the family member SMAD4. The association with SMAD4 is important for the translocation of this protein into the nucleus, where it binds to target promoters and forms a transcription repressor complex with other cofactors. This protein can also be phosphorylated by activin type 1 receptor kinase, and mediates the signal from the activin. Alternatively spliced transcript variants have been observed for this gene.

Recommended Dilutions

WB	1:4000 - 1:10000
IP	0.5 μg - 4 μg antibody for 200 μg - 400 μg extracts of whole cells
IF/ICC	1:200 - 1:15000
ChIP	2 μg antibody for 15 μg - 20μg of Chromatin
ELISA	Recommended starting concentration is 1 μg/mL. Please optimize the concentration based on your specific assay requirements. For high-ratio antibody dilutions (≥1:10000)，a sequential dilution method is strongly recommended to ensure measurement accuracy.

Immunogen Information

Gene ID
4087

Swiss Prot
Q15796

Immunogen
This information is considered to be commercially sensitive.

Synonyms
JV18; LDS6; CHTD8; MADH2; MADR2; JV18-1; hMAD-2; hSMAD2

Product Information

Source
Rabbit

Isotype
IgG

Purification
Affinity purification

Storage
Store at -20℃. Avoid freeze / thaw cycles.
Buffer: PBS with 0.09% Sodium azide,0.05% BSA,50% glycerol,pH7.3.

Contact

400-999-6126

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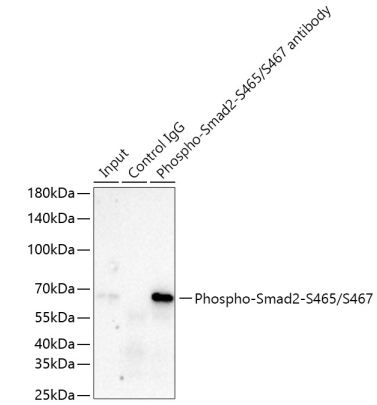
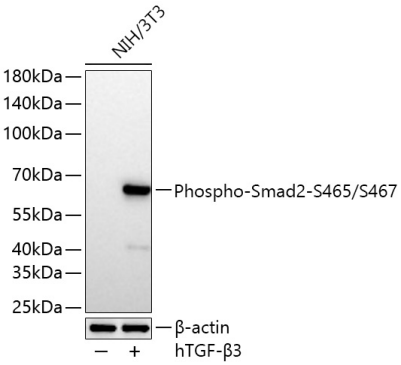
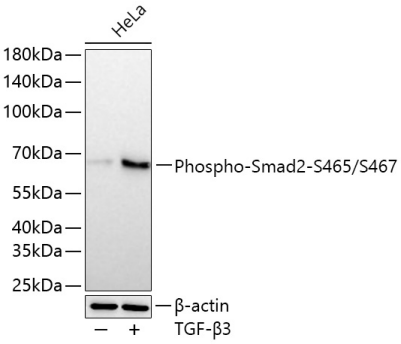
Validation Data



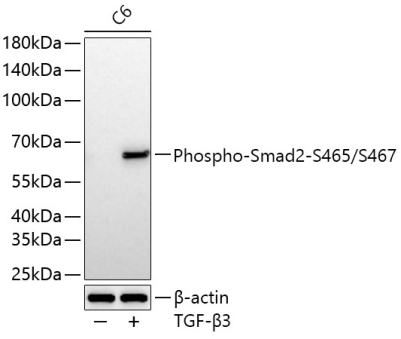
Immunoprecipitation of Phospho-Smad2-S465/S467 from 300 µg extracts of HaCaT cells treated with hTGF-β1 (7 ng/mL, 1 hour) was performed using 0.5 µg of Phospho-Smad2-S465/S467 Rabbit mAb (AP1654). Rabbit Control IgG (AC005) was used to precipitate the Control IgG sample. IP samples were eluted with 1x Laemmli Buffer. The Input lane represents 10% of the total input. Western blot analysis of immunoprecipitates was conducted using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) at a dilution of 1:10000.



Western blot analysis of lysates from NIH/3T3 cells using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) at 1:9000 dilution incubated overnight at 4℃. NIH/3T3 cells were treated with TGF-β3 (50 ng/mL) at 37℃ for 30 minutes.
Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.
Lysates/proteins: 30 µg per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 60 s.

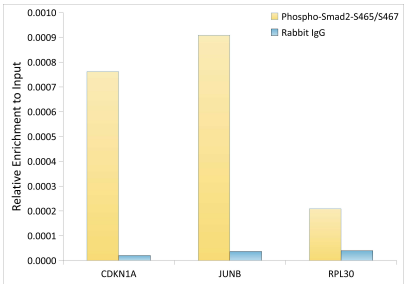


Western blot analysis of lysates from HeLa cells using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) at 1:9000 dilution incubated overnight at 4℃. HeLa cells were treated with TGF-β3 (50 ng/mL) at 37℃ for 30 minutes after serum starvation overnight.
Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.
Lysates/proteins: 30 µg per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 90 s.

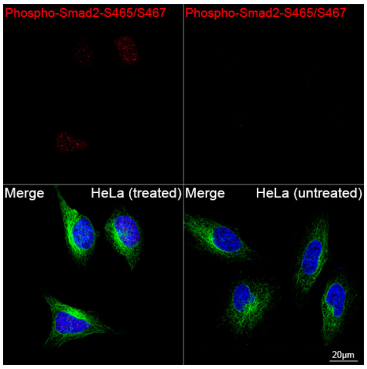


Western blot analysis of lysates from C6 cells using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) at 1:9000 dilution incubated overnight at 4℃. C6 cells were treated with TGF-β3 (50 ng/mL) at 37℃ for 30 minutes.
Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.
Lysates/proteins: 30 µg per lane.
Blocking buffer: 3% nonfat dry milk in TBST.
Detection: ECL Basic Kit (RM00020).
Exposure time: 90 s.

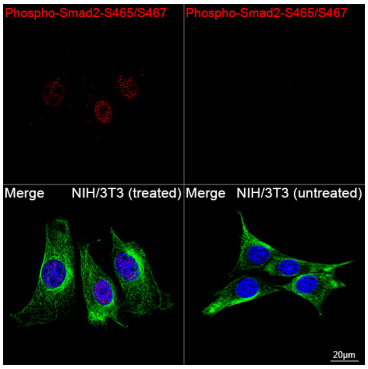
Validation Data



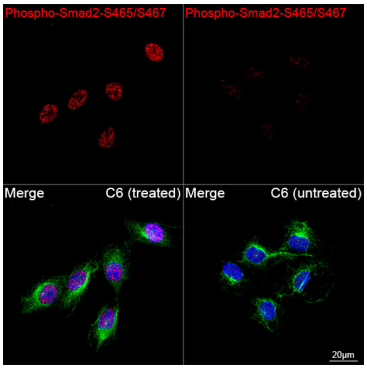
Chromatin immunoprecipitation was performed with 20 µg of cross-linked chromatin from HaCaT cells were treated with hTGF-β1 (7 ng/mL, 1 hour) , using 2 µg of Phospho-Smad2-S465/S467 Rabbit mAb (AP1654) and Rabbit IgG isotype control (AC042). The enrichment of immunoprecipitated DNA at different genomic loci was examined by quantitative PCR. The histogram compares the ratio of the immunoprecipitated DNA to the input at given loci.



Confocal imaging of HeLa cells (treated with TGFβ3) and HeLa cells (untreated) using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654, dilution 1:15000) followed by a further incubation with Cy3-conjugated Goat anti-Rabbit IgG (H+L) (AS007, dilution 1:500) (Red). The cells were counterstained with α-Tubulin Mouse mAb (AC012, dilution 1:400) followed by incubation with ABflo® 488-conjugated Goat Anti-Mouse IgG (H+L) (AS037, dilution 1:800) (Green). DAPI was used for nuclear staining (Blue). Objective: 100x.



Confocal imaging of NIH/3T3 cells (treated with TGFβ3) and NIH/3T3 cells (untreated) using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654, dilution 1:15000) followed by a further incubation with Cy3-conjugated Goat anti-Rabbit IgG (H+L) (AS007, dilution 1:500) (Red). The cells were counterstained with α-Tubulin Mouse mAb (AC012, dilution 1:400) followed by incubation with ABflo® 488-conjugated Goat Anti-Mouse IgG (H+L) (AS037, dilution 1:800) (Green). DAPI was used for nuclear staining (Blue). Objective: 100x.



Confocal imaging of C6 cells (treated with TGFβ3) and C6 cells (untreated) using Phospho-Smad2-S465/S467 Rabbit mAb (AP1654, dilution 1:200) followed by a further incubation with Cy3-conjugated Goat anti-Rabbit IgG (H+L) (AS007, dilution 1:500) (Red). The cells were counterstained with α-Tubulin Mouse mAb (AC012, dilution 1:400) followed by incubation with ABflo® 488-conjugated Goat Anti-Mouse IgG (H+L) (AS037, dilution 1:800) (Green). DAPI was used for nuclear staining (Blue). Objective: 100x.